Research Articles **Phytopharmacology Research Journal (PRJ) Open Access**

Received:12 December 2024 | Revised: 25 January 2024 | Accepted: 27January 2025 | Published online : 28 January 2025 | ISSN : 2959-9687

In vitro urease inhibitory screening of various extracts of *Calotropis procera* (Aiton) W.T.Aiton

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Funding information
Not applicable

Abstract

Natural products have traditionally been recognised as valuable sources of therapeutic compounds with various pharmacological actions. The objective of the current research was the investigation of phytochemical composition and in vitro urease inhibitory activity of the various solvent fractions of *Calotropis procera* (Aiton) W.T. Aiton. The shade-dried vegetal matter was dissolved in methanol in a Soxhlet apparatus, and the crude extract was fractionated into n-hexane, chloroform, ethyl acetate, methanol and butanol extracts.

Phytochemical analysis showed that the secondary metabolites (flavonoids, tannins, steroids, terpenoids, and phenolic compounds) are found mostly in the polar fractions. The jack bean urease (EC 3.5.1.5) assay was used to establish the urease inhibitory capacity of the fractions based on the use of thiourea as the standard. Among all fractions tested, the butanol fraction exhibited the strongest inhibitory activity (84.33% inhibition; IC_{50} = 34.45 ± 1.09 µg/mL), followed by methanol (80.40%; IC_{50} = 40.45 ± 1.05 µg/mL) and ethyl acetate (60.03%; IC_{50} = 72.15 ± 1.00 µg/mL), while n-hexane and chloroform fractions showed comparatively lower effects. The superior activity of the polar fractions suggests that flavonoids and phenolic constituents may play a key role in the enzyme inhibition.

These results indicate that *Calotropis procera* has great potential as a source of urease inhibitors in a natural selection, providing a typical application of it in traditional medicine and a possible direction for creating safer treatment methods in inhibiting urease-related diseases (peptic ulcers, urolithiasis, and Helicobacter pylori infection). This piece fits the United Nations Sustainable Development Goal 3 (Good Health and Well-being) as well, since it would support sustainable and plant-based drug discovery

KEYWORDS

Calotropis procera; Urease inhibitory activity; Phytochemical screening; Natural products; Secondary metabolites; Flavonoids; Phenolic compounds; Enzyme inhibition; Anti-ulcer potential; Sustainable Development Goal 3 (SDG 3)

1.0 INTRODUCTION

Nature, since ancient times, has been the keystone of drug discovery, and the overall biologically active compounds contained within nature represent a vast source of compounds that still find inspiration in modern pharmacotherapy [1]. Plants are, especially, an invaluable source of structurally diverse secondary metabolites, including alkaloids, terpenoids, flavonoids, tannins and phenolic acids, many of which have stunning bioactivities in a broad spectrum of pathological conditions [2]. The revival of interest in the natural products of the plants is not only due to therapeutic value but also due to their safety, their availability, and their eco-friendly nature [3]. Natural compounds over the last several decades have been supported by extensive phytochemical and pharmacological research to assert that they can target individual disease-relevant enzymes and receptors and provide safer alternatives to synthetic drugs [4].

Enzymes are among the several biological targets which

are essential in homeostasis, and their dysregulation is directly linked with different states of disease [5]. Urease (EC 3.5.1.5) is a nickel-dependent metalloenzyme, which is involved in the hydrolysis of urea into ammonia and carbon dioxide [6]. Though playing an important role in maintaining a healthy nitrogen metabolism, its excessive activity may cause pathological states like peptic and gastric ulcers, urinary tract infections, hepatic encephalopathy, and the development of kidney stones caused by infections. High levels of ammonia created by urease elevate local pH, destroying mucosal tissues and creating a good environment where microbes, especially Helicobacter pylori, can proliferate [7]. Hence, producing effective and safe urease inhibitors has taken centre stage in therapy. Nevertheless, most of the available synthetic urease inhibitors, like acetohydroxamic acid, are constrained by the negative side effects, and thus the need to identify plant-based urease inhibitors which can perform and at the same time be biocompatible is becoming a priority [8].

Calotropis procera (Aiton) W.T. Aiton, also called Sodom apple or Aak, is a perennial shrub of the family Apocynaceae and is commonly found in tropical and subtropical regions of Asia and Africa [9]. Some of the traditional medicine systems have utilized different components of this plant, such as the leaves, roots, latex, and flowers, to treat fever, asthma, leprosy and skin ailments [10]. Phytochemical research has indicated that C. procera has an abundance of alkaloids, flavonoids, terpenes, steroids, cardiac glycosides and phenolic compounds that synergistically make it have a variety of pharmacological effects, such as anti-inflammatory, analgesic, antimicrobial and antioxidant effects [11]. Although C. procera has been shown to have well-defined bioactivities, the urease inhibitory activity of the organism has not been significantly studied, especially in relation to the distributions of its bioactive metabolites across its various solvent-based fractions.

Phytochemical screening is an essential preliminary procedure to determine the chemical classes that produce bioactivity and to provide correlations between the particular metabolite and the observed pharmacological effects. As secondary metabolites (flavonoids, phenolics, and terpenoids) have been reported to bind enzyme active sites, a study of their connection with urease inhibition may prove new therapeutic opportunities. Previous studies on related medicinal plants of the Apocynaceae family indicate that the stronger enzyme inhibition is probably observed with mid-polar solvents, including methanol and butanol, because phenolic compounds are generally very high in concentration [12,13]. Therefore, the analysis of solvent-specific metabolite distribution in C. procera can be useful in assessing its biochemical possibilities.

Given the increasing demand for safe and plant-derived urease inhibitors, the given research was conducted to explore the phytochemical composition and in vitro urease inhibitory effect of different solvent fractions of Calotropis procera stem extracts. The proposed work will fill in the research gap that currently exists in this area, as it will determine the fractions that have the most promising enzyme-inhibitory properties and will connect such activities to their phytochemical components. In addition, the research is correlated with the United Nations Sustainable Development Goal (SDG) 3, which is Good Health and Well-being, as it encourages the investigation of the natural, sustainable resources to discover new drugs and develop plant-based methods of therapy [14].

2.0 MATERIALS AND METHODS

2.1 Plant collections

Fresh samples of Calotropis procera were obtained in the botanical garden of the Institute of Chemical Sciences, University of Peshawar, Pakistan. Taxonomic identification and authentication of the plant specimen were carried out by Mr Ghulam Jelani, Department of Botany, University of Peshawar, Pakistan. A voucher sample (UOS/Bot-139) was put in the departmental herbarium to be used later and for checking.

2.2 Extraction and Fractionation

2.2 Plant material in shade-dried *Calotropis procera* was rudely powdered and extracted with successive 48-hour extracts of a Soxhlet apparatus in methanol. The amassed solvent extract was filtered and concentrated by rotary evaporator at reduced pressure at 40ºC to obtain the crude methanolic extract. The extraction process was done using conventional phytochemical extraction conditions that have been described in the past [15-17].

2.3 Urease Inhibitory Activity

The jack bean urease (EC 3.5.1.5) urease inhibitory assay was used to determine the urease inhibitory activity of the n-hexane, chloroform, ethyl acetate, methanol and butanol extracts of *Calotropis procera*. Preparation of the reaction mixture was done in a 96-well microplate, and it contained 25 µL of enzyme solution, 55 µL of the phosphate buffer (100 mM, pH 8.2) containing urea as substrate (100 mM), and 5 µL of the test sample. The solution was left to incubate at 30 ºC.

The spectral photometric value of the enzyme activity was established by quantifying the quantity of ammonia produced through the indophenol procedure. Following the first incubation, 45 µL of phenol reagent (1% w/v phenol and 0.005% w/v sodium nitroprusside) and 70 µL of alkali reagent (0.5% w/v NaOH and 0.1% NaOCl containing active chlorine) were added to each of the wells individually. The samples were left to incubate further 50 minutes at room temperature, and the absorbance was measured at 630 nm with a microplate reader.

Each of the experiments was conducted three times, with a final reaction volume of 200 µL per well. Thiourea was employed as the reference standard inhibitor [18,19]. The degree of urease inhibition was determined by the following equation:

$$\text{Percent effect} = 100 - \frac{OD\ testwell}{OD\ control} \times 100$$

2.4 Statistical Analysis

The mean ± standard deviation (SD) of three independent replications (n = 3) was used to present all the results of the experiment. The data were compared with one-way analysis of variance (ANOVA), and the post hoc test was the Tukey test, which was used to analyze differences and establish the significance of differences between the groups of treatments. A p -value lower than 0.05 was taken as significant.

3.0 RESULTS AND DISCUSSION

The urease inhibitory ability of the different solvent fractions of *Calotropis procera* was measured to identify their urease-inhibitory capacity against the hydrolysis of urea through urease. The findings are indicated in Table 1, within which all the fractions displayed dose-dependent inhibitory activities, but the degree of inhibition differed according to the polarity of the solvent. The butanol fraction exhibited the best urease inhibitory effect (84.33%), with an IC 50 of 34.45 ±1.09 µg/mL, followed

by the methanol fraction (80.40% with an IC_{50} of 40.45±1.05 µg/mL). The ethyl acetate fraction was also significantly inhibited (60.03%; IC_{50} = 72.15 ± 1.00 µg/mL), but the chloroform and n-hexane fractions had a lower level of inhibition (32.09% and 14.59%, respectively). Thiourea, which was used as a reference standard, showed the most active result with an inhibition of 98.18% and an IC_{50} of 21.90 ± 0.04 µg/mL

Table-1. Urease inhibitory activity of hexane, chloroform, ethyl acetate, methanol, and butanol fractions of *Calotropis procera*

Extract/standard	Concentration (µg/mL)	% inhibition	IC_{50}
Hexane	0.2	14.59	-
Chloroform	0.2	32.09	-
Ethyl Acetate	0.2	60.03	72.15±1.00
Methanol	0.2	80.40	40.45±1.05
Butanol	0.2	84.33	34.45±1.09
Thiourea	0.2	98.18	21.90±0.04

This upward movement in the percentage of non-polar to polar fractions of activity indicates that the bioactive compounds that cause urease inhibition in *C. procera* consist mostly of polar or slightly polar compounds. It is possible to attribute the stronger performance of the butanol and methanol fractions to the abundance of polyphenolic compounds, in particular, flavonoids, tannin, and phenolic acids, which are linked with the known chelating effect on metal ions and interference with the active sites of an enzyme [20,21]. These compounds can bind to the nickel ions of the active site of urease to form a stable complex, which inhibits the catalytic mechanism of the urease protein.

The results obtained are consistent with the previous reports emphasizing the biological versatility of *C. procera*. The studies on phytochemicals have verified the presence of a variety of metabolites, such as cardenolides, terpenoids, flavonoids and glycosides, that play a role in its anti-inflammatory, antimicrobial, and cytoprotective effects [22]. It is important to note that the same trends were observed in the other plant species. As an example, Cassia fistula butanol fractions strongly inhibited urease, and this could be explained by the presence of phenolic-rich compounds [23], and Bauhinia purpurea methanolic extracts demonstrated similar inhibitory profiles [24]. These similarities reinforce the theory that polyphenolic compounds are at the centre stage of the urease inhibitory process of medicinal plants.

Mechanistically, flavonoids and tannins can inhibit urease either by directly binding to the nickel atoms in the active site or by disrupting the hydrogen-bonding network which must exist to bind a substrate [25]. These interactions may cause conformational changes in the tertiary structure of the enzyme, making it inactive. Conversely, the nonpolar fractions (n-hexane and chloroform) have low inhibitory potential, which suggests that lipophilic components, e.g. terpenoids and fatty acids, might not be able to bind to the enzyme's hydrophilic active site.

The pharmacological aspect of urease inhibition is of great relevance because the pathogenesis of various clinical conditions, such as gastric ulcers induced by Helicobacter pylori, hepatic encephalopathy, urinary tract infections, and others, involves excessive urease activity [26,27]. The fact that *C. procera* extracts, especially the butanol and methanol fractions, are capable of successfully inhibiting urease highlights the potential of such extracts in the creation of safe, natural alternatives to synthetic inhibitors, such as

acetohydroxamic acid, which is commonly linked to nephrotoxicity and teratogenicity [28,29].

Moreover, the current results indicate the biochemical richness of *C. procera* and give experimental evidence of its medicinal conventional applications. The activity observed may be synergistic in nature due to interactions of several phytochemicals found in the polar fractions, as opposed to the effect of one compound. It is a characteristic of natural extracts and frequently helps to achieve their therapeutic effect with low toxicity [30-32].

Generally, the findings indicate conclusively that the butanol and methanol extracts of *Calotropis procera* have potential urease inhibitory properties, confirming the ethnopharmacological status of the plant and its suitability in further natural product-derived drug development programs. Future studies are recommended to be on the bioassay-based isolation of active components, spectral analysis via LC-MS/MS and NMR and verification in vivo to ascertain safety and effectiveness.

4.0 CONCLUSION

The current research confirms Calotropis procera as an effective natural urease inhibitor. Significant inhibition of the enzyme was observed in the butanol and methanol fractions, indicating that phenolic and flavonoid compounds are the main causative agents of the activity. These results are a scientific justification of the usage of C. procera traditionally in the treatment of gastrointestinal and urinary tract system diseases associated with the presence of urease-producing pathogens. The research not only leads to the realization of the enzyme-modulating potential of C. procera, but it also helps to create safe and plant-based therapeutics that are in line with Sustainable Development Goal 3 (Good Health and Well-being). Further studies are required on purifying individual bioactive compounds and evaluating their pharmacokinetics and in vivo efficacy in order to promote their therapeutic utility.

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